Report for 2003KY17B: Biochemical and hormonal effects of incomplete site remediation: evaluating resident fish species

- Articles in Refereed Scientific Journals:
  - Brammell, B.F., D.J. Price, W.J. Birge, and A.A. Elskus, 2004, Apparent lack of CYP1A1 response to high body burdens in fish from a chronically contaminated PCB site, Marine Environmental Research , 58(2004), 251-255.
- Conference Proceedings:
  - Brammel, B.F., D.J. Price, W.J. Birge, and A.A. Elskus, 2004, Pollutant response in species inhabiting chronically contaminated habitats: Two varieties of resistance?, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentukcy Water Resources Research Institute, Lexington, Kentucky, 3-4.
  - Arzuaga, Xabier, and Adria Elsksus, 2004, AHR/CYP1A mediates PCB induction of teratogenesis and CYP1A, but not oxidative stress: An in ovo vetebrate model, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 51-52.
  - Price, D.J., and W.J. Birge, 2004, Determining bioavilable PCB fractions in freshwater stream sediments using PCB body burdens in fish sentinel monitors, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 53-54.

Report Follows

Problem and Research Objectives

Polychlorinated biphenyls (PCBs) are ubiquitous aquatic pollutants with significant toxic effects in both humans and fish, including altered reproduction, immunosuppression, carcinogenesis, and neurotoxicity. Significant levels of environmental PCBs in Kentucky have led to the posting of fish advisories in several Kentucky waterways (Kentucky Division of Water). The focus of the present study was the Town Branch-Mud River (TB/MR) system in Kentucky, a PCB-contaminated site currently under remediation. This work addresses several needs including the need to understand the impact of contaminants on higher organisms, to monitor the time course of recovery following contamination, and to evaluate the effectiveness of management efforts to improve water quality.

Results of our previous grant (2002KY1B) demonstrate that remediation of the PCB-contaminated TB/MR system has not been wholly successful. We found elevated levels of the biomarker enzyme, CYP1A1, in both gill and liver of trout caged in remediated sections of the TB/MR waterway, and preliminary data on PCB levels in sediments and resident fish confirmed the presence and bioavailability of these chemicals. Chronic exposure to PCBs can lead to the development of PCB-resistance in vertebrates, including fish, an effect associated with suppressed CYP1A1 expression. Exposure to PCBs can also disrupt endocrine systems in fish, including altering levels of thyroid hormone, a hormone involved in fish growth, reproduction, and the ability to cope with environmental stress. Both chemical resistance and endocrine disruption have population-level effects. Both CYP1A1 and UDP-GT, a key enzyme in TH metabolism and one that is altered by PCBs, are regulated through the aryl hydrocarbon receptor, suggesting there may be a common link between PCB effects on CYP1A1 and TH. We proposed to examine whether resident fish in the TB/MR have developed resistance to PCBs and whether resistant resident species also demonstrate resistance to disruption of thyroid hormone by PCBs

Methodology

Our first task was to make initial characterizations of resident populations in the TB/MR system as either PCB resistant or responsive, based on CYP1A1 expression level and PCB body burdens. In Fall 2002 and throughout 2003 we collected resident fish from the same TB/MR remediated, unremediated and reference sites we had used in our trout caging studies of 2002/3. Fishing Creek served as an additional reference site. Among the fish collected (green sunfish, longear sunfish, creek chub, yellow bullhead, rock bass, spotted bass, and common carp) were species known to develop resistance and those known to remain responsive when chronically exposed to PCBs [8, 19, 20]. We measured PCB body burdens and CYP1A1 activity in populations of these species from all three sites. Based on their CYP1A1 expression level relative to their PCB body burdens we classified resident populations as potentially resistant or responsive.

Our second task was to confirm PCB resistance and conduct studies to determine if PCB resistance was manifested by UDP-GT, another enzyme altered in resistant

mammals, and conferred resistance to the disruption of thyroid hormone by PCBs. To confirm resistance, we conducted laboratory-based PCB-challenge experiments and measured CYP1A1 response, a well-recognized test for resistance [4, 10]. Those populations that failed to respond to laboratory exposure to PCBs as measured by CYP1A1 induction were designated PCB-resistant and those that responded were designated PCB-responsive. Two of these resident populations, one resistant and one responsive, were then used in further laboratory PCB dose-response experiments and hepatic CYP1A1, UDP-GT and plasma thyroid hormone levels measured to evaluate possible correlations among these parameters and resistance to PCBs.

Principle Findings and Significance

Our first principle finding was that PCBs are present and bioavailable in the TB/MR system[1]. We found resident species in the remediated area of the Town Branch/Mud River system have PCB body burdens that are similar to, and in some cases well above, those known to induce CYP1A1 in other fish species [1, 11]. Highly elevated levels of PCBs remain in the sediments from the remediated section of Town Branch (up to 45 ppm total Aroclor dry wt basis, [6] and have been bioaccumulated to extraordinarily high levels by resident fish collected at this site (up to 98 ppm wet edible flesh, with a median concentration of 24 ppm; [6]. These levels are at the high end of those measured in fish from New Bedford Harbor, Massachusetts, considered one of the most highly contaminated PCB Superfund sites in the US. Median PCB levels in edible flesh ranged from 5.5 - 7.4 ppm for New Bedford Harbor flounder species up to 24 ppm median PCB for American eel [17]. PCB levels in fish from the unremediated section of the Mud River ranged from non-detectable up to 20 ppm (median 3.89 ppm), indicating that this site is also a significant source of PCBs for resident species. In comparison, Town Branch reference fish collected upstream of the remediated site had PCB body burdens that were up to 100 times lower than fish from the remediated site, ranging from non-detectable to < 3 ppm (median = 0.56 ppm).

Our second principle finding was that resident fish in PCB-impacted areas of the TB/MR system have developed resistance to at least some of the biological effects of PCBs. Based on a comparison of PCB body burdens and CYP1A1 levels in fresh-caught fish from reference and impacted sites, we initially characterized 6 populations (5 species) as likely to be PCB-resistant in the TB/MR system: longear sunfish and green sunfish from the TB remediated site, and yellow bullhead, spotted bass, rock bass, and longear sunfish from the unremediated MR site. These populations exhibited a lack of CYP1A1 induction, despite elevated body burdens of CYP1A1 inducing PCBs. The only species that appears not to have developed PCB-resistance in the PCB-impacted regions of the TB/MR system are creek chubs. In creek chubs from the unremediated TB site, elevated body burdens of PCBs were accompanied by elevated CYP1A1 expression, as would be expected in fish that retain responsiveness to the CYP1A1-inducing effects of PCBs and thus show the expected increase in CYP1A1 with increased PCB tissue levels .

[1] Some of these data were cited in our 2002/3 report.

To confirm our initial designations of PCB responsivity, and to identify two species for further enzyme and hormone analyses, we collected resident species from TB and MR and held them for 4 months in clean, dechlorinated water. This extensive depuration period was necessary to avoid confounding our challenge experiments by using fish with high PCB body burdens (half-life of PCBs in fish is 4 months [13]). For example, after an 18 week depuration period, PCB levels in edible flesh of yellow bullhead from the TB remediated site averaged 3.32 ± 0.92 (SE) ppm (n=5) compared to freshly caught longear sunfish, green sunfish and creek chub from this site whose body burdens ranged from 16 - 75 ppm. After the depuration period, we conducted laboratory-based PCB challenge experiments on TB yellow bullheads, TB green sunfish, TB creek chubs and MR spotted bass, and on corresponding species from reference sites. Because of the large numbers of fish involved (>100), experiments were conducted sequentially over several months.

As expected, yellow bullhead from reference, but not from the TB remediated site, responded to induction of CYP1A1 in the PCB laboratory challenge experiments. This confirmed our initial designations of responsive and resistant, respectively, for these resident populations, and is in keeping with the demonstrated ability of fish to develop resistance to halogenated hydrocarbons, including PCBs [9]. Green sunfish populations from both the reference and the unremediated TB sites failed to respond to laboratory PCB exposure. This finding was not unexpected as we had preliminary data on another *Lepomis* species, longear sunfish, that indicated that at least some members of this genus appear to have a natural, genetic resistance to CYP1A1 induction by PCBs [7] indicating that *Lepomis* species may not be useful biomonitoring organisms for PCBs.

Spotted bass proved difficult to work with in the laboratory, developing infections during the long-depuration period that resulted in high mortality rates. Seventy-five percent of the MR unremediated site fish, and 50% of the reference MR site fish died during depuration. After 14 weeks (rather than the full 16), we conducted PCB challenge experiments, but as expected, experimental mortality was high (33%) and the small replicate number for the depurated MR fish (n=2) made the results unreliable.

We are currently conducting PCB-challenge experiments to determine if creek chub, unlike yellow bullhead and green sunfish from the TB remediated site site, have failed to develop PCB resistance. Once we have confirmed the responsivity of the TB creek chub, we will commence the final biochemcial analyses for this project using the killifish, *Fundulus heteroclitus*. The purpose of this final experiment (conducted in Fall 2003) was to look at the relationship between CYP1A1, UDP-GT and thyroid hormone, and whether PCB resistance alters this relationship. The ability of halogenated aromatic hydrocarbons, including PCBs, to alter thyroid hormones, possibly by altering the activity of UDP-GT, has been reported in mammals [12, 16, 18]. We chose *Fundulus heteroclitus* for these experiments because PCB-resistant and -responsive populations have been well-characterized by our laboratory and others [2, 3, 10, 14, 15].

The current clean-up efforts in the TB/MR system provide an unparalleled opportunity to evaluate the effects of chronic chemical exposure and site remediation on local populations. The results of our present studies provide significant insights into current conditions in the TB/MR system and the response of resident fish to those conditions.

Specifically, the findings of our 2003/4 studies demonstrate that:

(1) it is likely that the duration of exposure, rather than the level of exposure, is what provokes the development of resistance to PCBs; MR fish developed resistance even though their PCB body burdens (1.2 - 7.4 ppm) were far lower than those of resistant TB fish (16 - 75 ppm).

(2) even under the same exposure regime, not all fish species appear to develop resistance. TB remediated site yellow bullhead, but apparently not creek chub (confirmation experiment in progress), have developed resistance to CYP1A1 induction by PCBs.

(3) the severity and extent of pollution problems in the TB/MR system, most significantly the presence of extraordinarily high levels of PCBs in the TB remediated site, suggest either insufficient remediation or continued input of PCBs to the system. We suggest that further remediation efforts be halted until the present source of PCBs to this system can be identified.

Chemical resistance in fish is a recently recognized phenomenon and almost nothing is known regarding the consequences in affected populations. Because resistance has 'costs', tolerant populations may demonstrate heightened susceptibility to further stressors (e.g. site remediation), resulting in unexpected population crashes during cleanup efforts. The final analyses of our 2003/4 study should reveal whether resident populations resistant to CYP1A1 induction are likely to be resistant to the hormone disrupting effects of PCBs and/or whether there is a tradeoff, whereby resistant fish lose the ability to retain normal hormone function.

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